Systematic review of purine analog treatment for chronic lymphocytic leukemia: lessons for future trials

CLL Trialists' Collaborative Group (CLLTCG)*

ABSTRACT

A systematic review of purine analogs revealed heterogeneity between trials in treatment effects on response and progression free survival, but not survival, perhaps partly due to variations in analytical methods. In addition, combination treatments required evaluation. Therefore, individual patient data were sought for all randomized trials in untreated chronic lymphocytic leukemia which involved a purine analog, but which did not include antibody therapies. Sixteen trials were found, addressing seven comparisons. Eight trials, with 2,753 patients, showed that single agent purine analog improved progression free survival (odds ratio=0.71; 95% confidence interval=0.63-0.79). Heterogeneity remained substantial. Three trials, with 1,403 patients, showed that progression free survival was further improved by the addition of cyclophosphamide (odds ratio=0.54; 0.47-0.62). Fewer data were available on the addition of other drugs to purine analog, and none showed clear benefit. Two trials, with 544 patients, suggested cladribine improved progression free survival compared to fludarabine (odds ratio=0.77; 0.63-0.95). No differences were seen in overall survival for any comparisons. In conclusion, purine analogs, particularly combined with cyclophosphamide, significantly improve progression free survival but not survival. Some groups, such as the elderly, may not see the same benefits and maximizing doses may be important for all treatments, including chlorambucil. Longer follow up, consistent definitions and detailed reporting of trials should be encouraged.

Key words: purine analog, combination therapy, CLL, review, cyclophosphamide.

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Introduction

The CLL Trialists' Collaborative Group was formed to bring together the results of all properly randomized CLL trials. In 1999, by combining individual patient data (IPD) from all trials that began before the end of 1990, the group demonstrated that the survival of early stage patients was not improved by chemotherapy, and that there was no evidence that combination chemotherapy was better than simple chlorambucil with or without prednis(ol)one.[1]

In 2006, a Cochrane Collaboration systematic review of single-agent purine analogs compared with alkylating agents was published.[2,3] This review used published data and included results from 5 trials but identified one other trial for which results could not be extracted from publications, and 3 more that had only recently closed. The primary end points in these trials varied from response to survival or progression free survival (PFS), and all three measures were analyzed in the review. No benefit of purine analogs was demonstrated in terms of survival but the numbers included were limited and data from the additional trials were needed before a firm conclusion could be drawn. Response rates were higher and PFS was longer with purine analogs. However, there was significant heterogeneity between the trials that might be largely or entirely due to differences between methods of response evaluation, PFS definitions, and analytical methods.

With the completion and publication of the additional trials, it was agreed that the collaborative group would address this question using IPD, and also investigate combination treatments that included purine analogs. Antibody therapies were excluded as the trials were too recent and data were not yet available. Use of IPD would allow examination of differences in the timings of response evaluations and the use of a more uniform definition of PFS.

Design and Methods

All randomized trials of active treatment comparisons in untreated CLL which involved at least one treatment arm including a purine analog, and which began in 2004 or before, were included, with the exception of those involving an antibody therapy, such as rituximab or alemtuzumab.

The Clinical Trial Service Unit has established a database of randomized trials in leukemia, identified by periodic searches of electronic databases including MEDLINE, EMBASE, meeting abstracts and clinical trial registration databases. For this review, additional review

**CLLTCG Participating groups and trialists are listed in the "Appendix".*
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Correspondence: Sue Richards, CLLTCG secretariat, Richard Doll Building, University of Oxford, Old Road Campus, Roosevelt Drive, Oxford OX3 7LF, UK. Phone: international +44.01865743863. Fax: international +44.01865743986. E-mail: sue.richards@ctsu.ox.ac.uk

articles, meeting abstracts (ASH, EHA, IWCLL) and reference lists of published trials were hand searched. Principal investigators from the identified trials were invited to join the collaborative group, to provide preliminary data, and to attend a meeting in 2007 at which preliminary results were presented (*Online Supplementary Appendix)*. These, and other experts in the field, were consulted to ensure completeness of the list of relevant trials.

Information on each trial was sought from protocols, publications and the trialists themselves. As well as details of eligibility criteria and treatments (including duration and protocol defined crossovers), methods of randomization, definition and timing of response assessments, definition of disease progression and of PFS used in any reports, and whether the trial reached its accrual target or stopped early (with reason if relevant) were collected. The project was approved by the Oxford Ethics Committee (OXTREC).

For each trial, data were requested for each individual patient on patient and disease characteristics, treatment allocation and outcomes (*Online Supplementary Table S1*). Information recorded on adverse effects varied greatly between trials and was not collected as IPD. Data on some common toxicities were obtained from publications.

Data for each trial were checked for consistency (range checks including consistency with eligibility criteria, dates in order, stage calculated from variables supplied against specified stage) and balance of treatment allocations over chronological time, over sex, stage, age, and by length of follow up. Queries, including missing variables, as well as tables of numbers in different groups by treatment allocation for checking, were sent to the principal trial investigators and amendments were made to the data according to their response.

All analyses only compared patients with others in the same trial. In order that no bias was introduced by comparing patients on a particular treatment with others who could not have been allocated that treatment, plots of randomization over the course of each trial were drawn. If the balance between arms was not maintained over the whole period of the trial and the trialists stated this was due to a major modification such as the early closure or late introduction of one arm of a trial, the trial was split into two parts; these were analyzed separately and the results summed. When a trial included more than 2 arms, and more than one comparison was relevant to a particular question, results are displayed for each comparison but the overall result is adjusted so that patients are only counted once.

Details of statistical methods used are described in the *Online Supplementary Appendix*. The primary analyses were of good response (complete or nodular partial), any response, PFS and overall survival. Good response analyses were repeated with nodular partial response not counted as good response to see whether this affected conclusions. Responses were analyzed as binary variables, while PFS and survival were treated as time to event variables. PFS analyses counted lack of response to first-line treatment, any progression and death as events. In trials in which no assessment date was recorded for the non-responders, these were counted as having an event on the earliest date of death, date of last follow up or expected date of response measurement according to the protocol. As date of progression was not supplied for one trial, but published results were available for disease free survival (DFS), excluding non-responders, this outcome was additionally used in analyses including this trial using published data from it.[4]

Subgroup analyses were pre-planned by sex, age (<60, 60-69, ≥70 years), stage, IGHV (mutated or unmutated), 17p13 deletion or not, and by year of follow up. As some trials used the Binet and others the Rai staging system, stage was divided into two groups with Binet stage C or Rai stages 3 or 4 classified as high and other stages as low. Analyses of response and survival from second-line treatment were planned, but data proved to be too sparse to allow an analysis to be made. In the light of the emerging prognostic relevance of Beta-2 microglobulin, it was agreed at the collaborators meeting that this factor should be added to the subgroup analyses. 11q deletion was added later. Subgroup analyses were only examined where data were available for more than one trial.

Where there was substantial heterogeneity between trials, the possible reasons were explored.

Results

Sixteen eligible trials were found. These are listed in Table 1. One additional trial[21] was excluded because it included only previously treated patients. The CLL101 trial included treated patients but only the untreated patients were included in these analyses. Randomization methods, definitions of response and progression used, and treatment duration, including protocol defined crossovers, are described in *Online Supplementary Table S2*. Only 2 trials (LRF CLL4, NCI Egypt) used nodular partial as a response category.

Online Supplementary Table S3 shows the trial sizes, length of follow up and patients' characteristics for all 13 trials which supplied individual patient data (IPD). Median follow up ranged from 2 to 12 years. Male patients accounted for 63-74% of study subjects and most male and female patients were under 70 years of age. Only a small subset of trials were able to supply data on beta-2 microglobulin, IGHV mutation status, and 17p13 and 11q deletion.

The comparisons addressed by one or more trials were: i) single agent purine analog (PA) *versus* alkylating agent based treatment; ii) addition of cyclophosphamide to PA; iii) PA plus cyclophosphamide *versus* alkylating agent based; iv) addition of chlorambucil to PA; v) addition of epirubicin to PA; vi) addition of mitoxantrone to PA; vii) cladribine *versus* fludarabine. Some trials randomized between more than two arms and hence addressed more than one question.

There were no significant differences (trend or heterogeneity test $P>0.1$) between the treatment effects on response, PFS or survival seen in different subgroups by sex, age, stage, beta-2 microglobulin, or 17p13 or 11q23 deletion for any of the comparisons, except in the cases mentioned below (a and b), but some subgroup numbers were very limited. Nor was there generally evidence for a different effect on PFS by year of follow up.

a) Single agent PA versus *alkylating agent based treatment*

Ten trials addressed this question. This included one trial (Tirana) which was not identified in the Cochrane review; the only publication for this trial was an EHA abstract.[18] IPD were not available and only information on overall response was given in the abstract. The Italian multicenter trial[8] also only reported on response in an abstract. These trials are excluded. Individual patient data were available for 8 trials, including 2,753 patients. One 3-arm trial (Scandinavian/Australian) contributed to 2 comparisons: fludarabine *versus* chlorambucil and cladribine *versus* chlorambucil.

Response data were available for all 8 trials and for 2,596 patients. The chance of obtaining a good response, or any response, was higher with PA compared with alkylating agents (risk ratio (RR)=1.81, 95% CI=1.59-2.08, $P<0.00001$; RR=1.20, 95% CI=1.13-1.26, $P<0.00001$, respectively) (Table 2, *Online Supplementary Table S4*). There was substantial heterogeneity between trials ($P<0.001$) both for good ($I^2=78\%$) and for any response (I2=83%). As not all trials

included nodular partial as a response category, this analysis was repeated excluding this from the good response category. This marginally increased the relative risk (RR=1.93; 95% CI=1.66-2.25) but heterogeneity remained (I^2=83%).

The trials were grouped according to whether they used fludarabine or cladribine, and by type of alkylating based regimen: i) chlorambucil with or without prednisolone; ii) cyclophosphamide, doxorubicin, plus prednisolone, with or without vincristine. In spite of the small numbers of trials in each subgroup, heterogeneity remained.

The EORTC trial used a different type of alkylating regimen with chlorambucil given at a dose of 10mg/m^2 every day for 18 weeks (maximum cumulative dose 1,260 mg/m^2), toxicity-tailored, resulting in about 60% of this dose administered (756 mg/m^2). All the other trials used intermittent treatment for between one and ten days every four weeks. Exclusion of this trial did not remove the heterogeneity, which may be partly due to differences in timings and recording of response (*Online Supplementary Appendix: Results*).

PFS analyses included 7 trials (8 comparisons) involving 1,816 patients. One trial, FRE-CLL-90, did not provide dates of progression and so did not contribute to these analyses. Only the LRF CLL4 and EORTC 06916 trials provided dates

Table 1. Trial details (start year, eligibility, treatments).

Trial name	Start year	Eligibility Criteria	Treatments
CLL101[5]	1990	Stage B,C Age 18-75 years	1: Fludarabine 25 mg/m^2 i.v. d1-d5/4wk x 6 2: (Cyclo 750 mg/m^2 i.v. d1 + Doxo 50 mg/m^2 i.v. d1 + Pred 40mg/m^2 oral d1-d5)/4wk x 6
FRE-CLL-90[4]	1990	Stage B, C Age <75 years	1: Fludarabine 25 mg/m^2 i.v. d1-d5 x 6 2: (Cyclo 750 mg/m^2 i.v. d1 + Doxo 50 mg/m^2 i.v. d1 + Pred 40mg/m^2 oral d1-d5)/4wk x 6 3: (Cyclo 300 mg/m^2 oral d1-d5 + Doxo 25 mg/m^2 i.v. d1 + Vinc 1mg/m^2 i.v. d1 + Pred 40 mg/m^2 oral d1-d5)/4wk x 6
CLB 9011[6]	1990	Stage B,C	1: Fludarabine 25 mg/m^2 i.v. d1-d5/4wk x max 12 2: Chl 40mg/m^2 oral d1/4wk x max 12 3: (Fludarabine 20 mg/m^2 i.v. d1-d5 + Chl 40mg/m^2 oral d1)/4wk x max 12
EORTC 06916[7]	1993	Advanced disease Age 18-80 years	1: Fludarabine 25 mg/m^2 i.v. d1-d4/3wk x 6 2: Chl 10mg/m^2 oral daily x 18wk (toxicity-tailored)
Italian Multicenter[8]	1994	Rai intermediate or high risk	1: Fludarabine 25 mg/m^2 i.v. d1-d5/4wk x 3-9 2: (Chl 30mg/m^2 oral d1,d15 + Pred 40 mg/m^2 i.m. d1-d5, d15-d19)/4wk x 3-9
PALG CLL1[9,10]	1995	Stage III or IV or progressive 0, I or II	1: (Cladribine 0.12 mg/kg i.v. d1-d5 + Pred 30 mg/m^2 oral d1-d5)/4wk x 3-6 2: (Chl 12mg/m^2 oral d1-7 + Pred 30 mg/m^2 oral d1-d7)/4wk x 3-6
Scandinavian/ Australian[11]	1997	Stage B, C or progressive A Age 18-75 years	1: Fludarabine (25 mg/m^2 i.v. or 40 mg/m^2 oral) d1-d5/4wk x 6 2: Cladribine (5 mg/m^2 sc or i.v. or 10 mg/m^2 oral) d1-d5/4wk x 6 3: Chl 10mg/m^2 oral d1-d10/4wk x 6
SHG[12]	1997		1: Fludarabine 25 mg/m^2 d1-d5/4wk x max 6 2: (Fludarabine 25 mg/m^2 d1-d5 + Eprubicin 25 mg/m^2 d4,d5)/4wk x max 6
LRF CLL4[13]	1999	Stage B, C or progressive A	1: Fludarabine (25 mg/m^2 i.v. or 40 mg/m^2 oral) d1-d5/4wk x max 6 2: ((Fludarabine 25 mg/m^2 i.v. + Cyclo 250 mg/m^2 i.v.) d1-d3 or (Fludarabine 24 mg/m^2 oral) + Cyclo 150 mg/m^2 oral) d1-d5)/4wk x max 6 3: Chl 10 mg/m^2 oral d1-d7/4wk x max 12
Intergroup E2997[14]	1999	Requiring chemo Age ≥18 years	1: (Fludarabine 20 mg/m2 i.v. d1-d5 + Cyclo 600 mg/m2 i.v. d1)/4wk x max 6 2: Fludarabine 25 mg/m2 i.v. d1-d5/4wk x max 6
PALG CLL2[15]	1999	Rai stage III, IV or progressive 0, I or II Age ≥18 years	1: (Cladribine 0.12 mg/kg i.v. d1-d3 + Cyclo 650 mg/m^2 i.v. d1 +Mitoz 10 mg/m^2 d1)/4wk x max 6 2: (Cladribine 0.12 mg/kg i.v. d1-d3 + Cyclo 650 mg/m^2 i.v. d1)/4wk x max 6 3: Cladribine 0.12 mg/kg i.v. d1-d5/4wk x max 6
GCLLSG CLL4[16]	1999	Stage B, C or progressive A Age ≤ 65 years	1: (Fludarabine 30 mg/m^{2}i.v. + Cyclo 250mg/m^2 i.v.)d1-d3/4wk x 6 2: Fludarabine 25 mg/m^2 i.v. d1-d5/4wk x 6
GCLLSG CLL5[17]	1999	Stage C or symptomatic A or B Age 65-80 years	1: Fludarabine 25 mg/m^2 i.v. d1-d5/4wk x max 6 2: Chl 0.4 mg/kg, escalating to 0.8m/kg, oral d1, d15/4wk x max 12
Tirana[18]	2001?	Stage B or C Age >18 years	1: Fludarabine 15 mg/m^2 i.v. d1-d5 2: Cyclo + Vinc + Pred
NCI Egypt[19]	2001	Rai stage III, IV or progressive I, II Age <65 years	1: (Fludarabine 25 mg/m^2 i.v. + Cyclo 250 mg/m^2 i.v.)d1-3/3wk x 3-6 2: (Cyclo 400 mg/m^{2}i.v. d1-d3 + Vinc 1.4 mg/m^2 d1 + Pred 100 mg/m^2 oral d1-d5)/3wk x 3-6
PALG CLL3[20]	2004	Progressive & symptomatic	1: (Cladribine 0.12 mb/kg i.v. + Cyclo 250 mg/m^2 i.v.)d1-d3/4wk x max 6 2: (Fludarabine 25 mg/m^2 i.v. +Cyclo 250 mg/m^2 i.v.) d1-d3/4wk x max 6

d: day, wk: weeks, Cyclo: cyclophosphamide, Doxo: doxorubicin, Pred: prednisolone, Chl: chlorambucil, Vinc: vincristine.

of response assessment for the non-responders.

PFS was better with PA with about 30% reduction in the event rate (OR=0.71; 95% CI=0.64-0.79; *P*<0.00001) (Figure 1A). There was an absolute difference in PFS of 4.7% at five years (*Online Supplementary Figure S1*). There was substantial overall heterogeneity between trials (*P*<0.0001). Application of random effects model gave OR=0.64; 95% CI=0.52-0.87; *P*=0.003. Heterogeneity was significant between subgroups but also within the fludarabine *versus* chlorambucil (FvC) subgroup. In addition to the protocol differences described (*Online Supplementary Table S2*) treatment intensity varied (Table 3). The largest effect in the FvC subgroup was seen in the CLB-9011 trial, which had the highest maximum cumulative protocol dose of fludarabine and the lowest of chlorambucil; the effect in the other trial was much less (OR=0.92; 95% CI=0.80-1.05; *P*=0.2).

Progression dates were not supplied for the FRE-CLL90 trial so that it could not be included in PFS analyses. It was possible to extract data on progression free survival from the FRE-CLL90 trial publication but this referred only to responders. So this was combined with DFS analyses (which excluded non-responders) of IPD data from the other trials. Overall DFS was better with PA (OR=0.81; 95% CI=0.72-0.90; *P*=0.0001) (Figure 1B). Heterogeneity between trials remained (*P*<0.00001) but not between subgroups, as the effect in the FRE-CLL90 trial, which compared fludarabine with CAP and CHOP, was substantially less than that in CLL101, which included only CAP. Random effects meta-analysis gave OR=0.74; 95% CI=0.58-0.95; *P*=0.02.

The effect on PFS of PA compared with alkylating agents was larger in the IGHV unmutated subgroup than in the mutated group (heterogeneity *P*=0.04) (Figure 2). There was a suggestion that the effect diminished with year of follow up but this was not significant (*P*=0.07).

There was no improvement in survival with PA (OR=0.93; 95% CI=0.85-1.03; *P*=0.2; Figure 1C). The survival difference with PA was also larger in the unmutated subgroup (heterogeneity *P*=0.04). A test for trend (*P*=0.03) suggested that the effect diminished with age, with no evidence of any benefit, and possibly worsened survival, in the 70 years and over age group (OR=1.14; 99% CI=0.88-1.49).

Only 4 trials (EORTC-06916, LRF CLL4, GCLLSG CLL5, PALG CLL1) provided data on second-line treatments. As one might expect, more patients were reported as receiving second-line treatment in the alkylating agents arms (61%, 58%, 60%, 28%) compared with the PA arms (49%, 51%, 35%, 40%). In the alkylating arms, the proportions of patients with second-line PA, single agent or combined, were 12%, 43% 34% and 35%, while in the PA arms the proportions receiving Chl were 45%, 0%, 3% and 16%.

b) Addition of cyclophosphamide to PA

Three trials compared fludarabine plus cyclophosphamide with fludarabine alone, and one compared cladribine plus cyclophosphamide with cladribine. Data were available for all 4 trials, with 1,403 patients. Response rates were substantially higher with the addition of cyclophosphamide (RR=1.64, 95% CI=1.37-1.96, *P*<0.0001 for good response, RR=3.09, 95% CI=2.24-4.26 if nodular partials were excluded, RR=1.15, 95% CI=1.09-1.21, *P*<0.0001 for any response; Table 2 and *Online Supplementary Table S4*). PFS was substantially improved by the addition of cyclophosphamide, with a halving of the event rate (OR=0.54, 95% CI=0.47-0.62, *P*<0.00001) (Figure 2). This resulted in an absolute improvement of 20% at five years (*Online Supplementary Figure S2*). In spite of this large difference, there was no significant effect on survival (OR=0.97, 95% CI=0.81-1.16, *P*=0.7) (Figure 3B). Heterogeneity was seen between the effect seen in the fludarabine trials and the one trial that used cladribine in terms of good response, with or without nodular partial, (*P*=0.003) and PFS (*P*=0.05), with a larger effect in the fludarabine trials. There was a suggestion of greater benefit in higher stage patients for PFS

Table 2. Relative treatment effects on response rates: relative risk (99% or 95% confidence interval[b]).

	Good response	Any response
Purine analog *vs.* alkylating agent based		
Fludarabine *vs.* chlorambucil		
CLB-9011	5.08 (1.80-14.35)	1.72 (1.27-2.32)
EORTC 06916	0.71 (1.42-2.10)	0.89 (0.74-1.09)
Scand/Aust[11a]	0.82 (0.18-3.68)	1.13 (0.81-1.57)
LRF CLL4	1.57 (1.15-2.16)	1.10 (0.97-1.25)
GCLLSG-5	14.49 (0.34-621.88)	1.47 (1.13-1.90)
Fludarabine *vs.* cyclophosphamide+doxorubicin+prednisolone+/-vincristine		
CLL101	1.50 (0.54-4.20)	1.23 (0.93-1.63)
FRE-CLL90	1.69 (1.31-2.17)	1.07 (0.96-1.20)
Cladribine+/-prednisolone *vs.* Chlorambucil+/-prednisolone		
PALG CLL1	4.31 (1.99-9.36)	1.47 (1.15-1.87)
Scand/Aust[11a]	1.37 (0.37-5.14)	1.18 (0.85-1.63)
Total	**1.81 (1.59-2.08) *P*<0.0001**	**1.20 (1.13-1.26) *P*<0.0001**
Addition of cyclophosphamide to single agent purine analog		
LRF-CLL4	1.44 (1.10-1.89)	1.17 (1.05-1.31)
E2997	5.26 (1.77-15.65)	1.19 (0.97-1.46)
G-CLL4	3.22 (1.26-8.26)	1.14 (1.03-1.26)
P-CLL2	1.08 (0.66-1.77)	1.11 (0.95-1.29)
Total	**1.64 (1.37-1.96) *P*<0.0001**	**1.15 (1.09-1.21) *P*<0.0001**
Fludarabine +cyclophosphamide *vs.* alkylating agents		
LRF CLL4	2.26 (1.73-2.97)	1.29 (1.17-1.42)
NCI Egypt	2.59 (0.92-7.28)	1.38 (0.81-2.37)
Total	**2.29 (1.87-2.80) *P*<0.0001**	**1.30 (1.21-1.40) *P*<0.0001**
Addition of chlorambucil to single agent purine analog		
CLB9011	0.77 (0.40-1.49) *P*=0.3	0.94 (0.72-1.23) *P*=0.6
Addition of mitoxantrone to cladribine plus cyclophosphamide		
P-CLL2	1.40 (0.92-2.13) *P*=0.04	0.94 (0.82-1.08) *P*=0.3
Cladribine *vs.* fludarabine		
Scand/Aust[11]	1.67 (0.41-6.79)	1.04 (0.78-1.40)
P-CLL3	0.97 (0.74-1.27)	1.02 (0.93-1.13)
Total	**1.00 (0.82-1.23) *P*=1.0**	**1.03 (0.95-1.11) *P*=0.5**

[a]*The Scand/Aust trial*[11] *contributes only once to the total using fludarabine + cladribine arms* versus *chlorambucil.* [b]*99% confidence intervals for individual trials, 95% for subtotals and totals.*

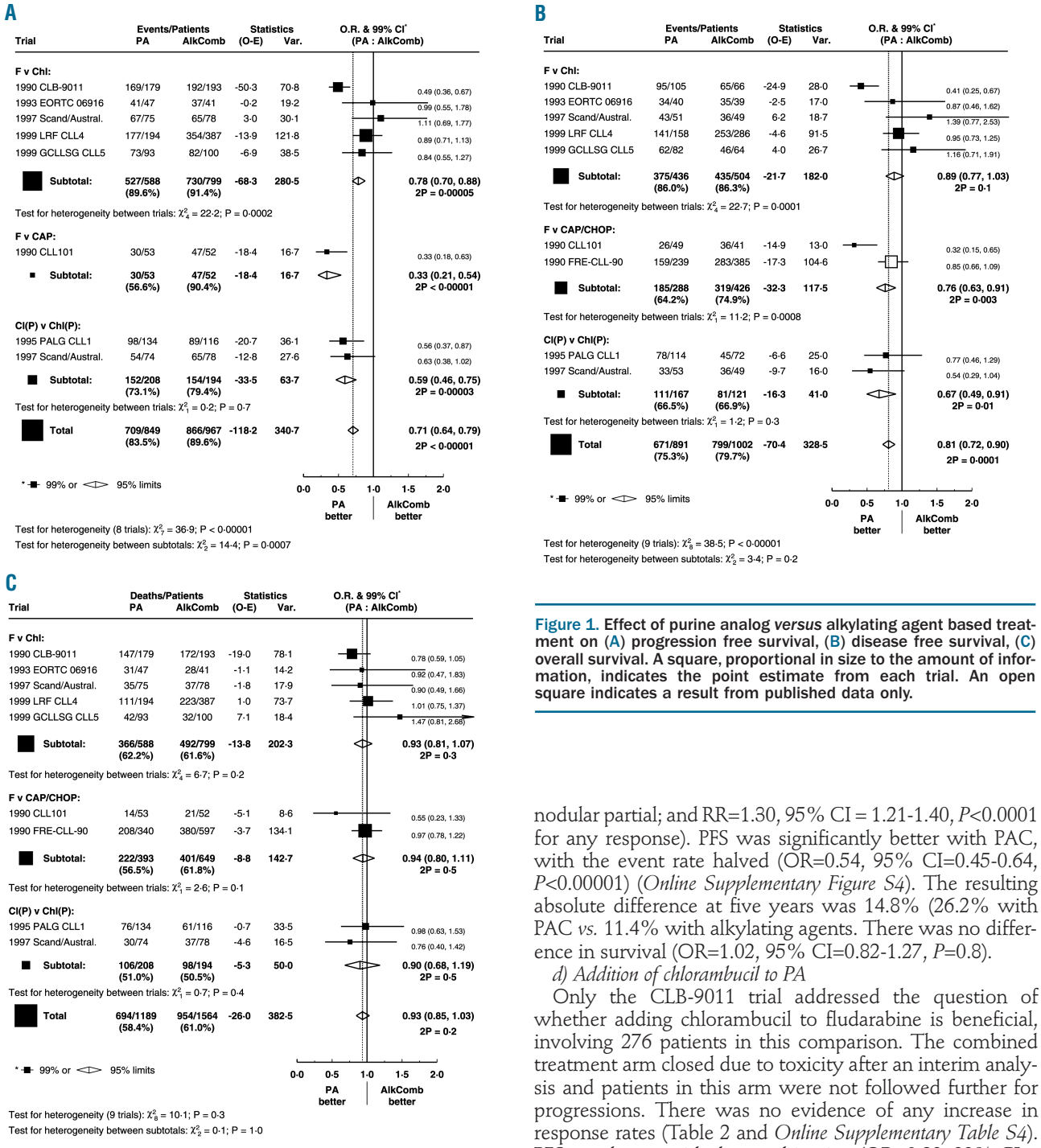


Figure 1. Effect of purine analog *versus* alkylating agent based treatment on (A) progression free survival, (B) disease free survival, (C) overall survival. A square, proportional in size to the amount of information, indicates the point estimate from each trial. An open square indicates a result from published data only.

(heterogeneity *P* value=0.02) (*Online Supplementary Figure S3*) and that the effect diminished with year of follow up (*P*=0.03). Although neither were significant, survival was better with cyclophosphamide in the higher stage group and worse in the lower stage subgroup (heterogeneity *P*=0.03).

c) PA plus cyclophosphamide versus *alkylating agent based*

Two trials (NCI Egypt and LRF CLL4, involving 645 patients) compared fludarabine plus cyclophosphamide (PAC) with alkylating agent therapy (chlorambucil alone, or cyclophosphamide, vincristine plus prednisolone). Data were available for both of these. Response rates were much higher with PAC (RR=2.29, 95% CI=1.87-2.80, *P*<0.0001 for good response; RR=5.07, 95% CI=3.44-7.50 excluding nodular partial; and RR=1.30, 95% CI = 1.21-1.40, *P*<0.0001 for any response). PFS was significantly better with PAC, with the event rate halved (OR=0.54, 95% CI=0.45-0.64, *P*<0.00001) (*Online Supplementary Figure S4*). The resulting absolute difference at five years was 14.8% (26.2% with PAC *vs.* 11.4% with alkylating agents. There was no difference in survival (OR=1.02, 95% CI=0.82-1.27, *P*=0.8).

d) Addition of chlorambucil to PA

Only the CLB-9011 trial addressed the question of whether adding chlorambucil to fludarabine is beneficial, involving 276 patients in this comparison. The combined treatment arm closed due to toxicity after an interim analysis and patients in this arm were not followed further for progressions. There was no evidence of any increase in response rates (Table 2 and *Online Supplementary Table S4*). PFS was better with the combination (OR=0.89, 99% CI = 0.61-1.28, *P*=0.4) (*Online Supplementary Figure S4*), while survival was worse (OR=1.12, 99% CI=0.79-1.57, *P*=0.4), but these differences were without statistical significance.

e) Addition of epirubicin to PA

Only the SHG trial examined whether adding an anthracycline, epirubicin, to fludarabine was beneficial. Data were not available for this trial. Published results report improved response rates (RR=3.16, 99% CI=1.12-8.98, *P*=0.004 for good response; RR=1.21, 99% CI=0.97-1.49, *P*=0.02 for any response). Survival analyses have not been reported.

f) Addition of mitoxantrone to PA

The PALG CLL2 trial examined adding mitoxantrone to cladribine plus cyclophosphamide involving 373 patients. There was no evidence of an effect on overall response rate,

Table 3. Treatment intensity in purine analog *versus* alkylating agent based trials.

	Purine analog					Chlorambucil/cyclophosphamide				
Fludarabine trials	mg/m²	days/ 4 wks	max courses	max dose per 4 wks	max cum dose	mg/m²	days/ 4wks	max courses	max dose per 4 wks	max cum dose
LRF CLL4[13]	25 iv or 40 oral	1-5	6	125 iv or 200 oral	750 iv or 1200 oral	Chl: 10	1-7	12	70	840
Scand/Aust[11] Fludarabine	25 iv or 40 oral	1-5	6	125 iv or 200 oral	750 iv or 1200 oral	Chl: 10	1-10	6	100	600
CLB-9011[6]	25 iv	1-5	12	125 iv	1500 iv	Chl: 40	1	12	40	480
GCCLLSG CLL5	25 iv	1-5	6	125 iv	750 iv	Chl: 0.4-0.8 mg/kg (~16-32)[a]	1,15	12	48-96	576-1152
EORTC 06916[7]	25 iv /3wk	1-4	6 (4.5 mths)	133.3 iv	600 iv	Chl: 10	daily	4.5 mths	280	1260[b]
FRE-CLL-90[4]	25 iv	1-5	6	125 iv	750	CAP C: 750 iv CHOP C: 300 oral	1 1-5	6 6	750 1500	4500 9000
CLL101[5]	25 iv	1-5	6	125 iv	750	CAP C: 750 iv	1-5	6	750	4500
Cladribine trials										
Scand/Aust[11] Cladribine	5 sc or iv or 10 oral	1-5	6	25 iv or 50 oral	150 sc or iv or 300 oral	10	1-10	6	100	600
PALG CLL1	0.12mg/kg iv (~7.2)	1-5	6	36 iv	216	12 (+Pred)	1-7	6	84	504

Approximate conversion of mg/kg to mg/m² obtained by multiplying by 40. [a]Dose increased according to tolerability. Maximum achieved in 20%. [b]Toxicity tailored doses. Report states 63% of dose can be safely administered.

but there were more good responses (RR=1.40, 99% CI=0.92-2.13, *P*=0.04). However, PFS was worse (OR=1.29, 99% CI=0.87-1.92, *P*=0.1) (*Online Supplementary Figure S4*) as was survival (OR=1.37, 99% CI=0.79-2.37, *P*=0.1), although these differences were without statistical significance.

g) Cladribine versus *fludarabine*

Two trials involving 544 patients compared cladribine with fludarabine, one as single agent, and one in combination with cyclophosphamide. There was no statistical difference in response rates (OR=1.00, 95% CI=0.82-1.23, *P*=1.0 for overall response) and survival (OR=0.79, 95% CI=0.59-1.05, *P*=0.1). PFS was better with cladribine (OR=0.77, 95% CI=0.63-0.95, *P*=0.01) but there was significant heterogeneity between trials for this outcome (*P*=0.008).

Discussion

For many decades, alkylators were the mainstay of CLL therapy and the addition of other chemotherapeutic agents did not appear to improve outcome. In 1990, purine analogs were introduced into clinical trials and they quickly demonstrated improved response rates,[22] but none of the trials was able to demonstrate an overall survival benefit. A Cochrane Review based on published trial data was also not able to detect any overall survival benefit but a significant degree of heterogeneity was found.[2,3] To overcome the limitations of meta-analyzing data derived from published trials, a collaborative IPD analysis was conducted integrating individual patient data from all trials to examine whether, with larger patient numbers available, any clinically worthwhile survival differences could be detected, and whether apparently discrepant trial results might be, at least partially, explained by variations in analytical methods.

IPD analyses often report weaker treatment effects than meta-analyses based on tabulated data from publications.[23] In our study, however, the magnitude of the treatment effects determined for purine analogs was comparable to those of previous reports substantiating their activity in CLL. In addition, the availability of individual patient data allowed for subgroup analyses according to age, sex, stage, beta-2-microglobulin, IGHV mutational status and presence of unfavorable cytogenetics, i.e. del(17p13) and del(11q23), in a significant subset of patients. However, concerning quantity, quality and duration of responses, no major differences were found for any of these subgroups except for the IGHV mutational status indicating a larger treatment effect for purine analogs in unmutated patients (*P*=0.04, n=593).

Although 70% of CLL patients are over 65 years of age,[24] most trials enrolled mainly younger patients with a median age of approximately 60 years. Hence, the majority of CLL patients have been greatly underrepresented in clinical trials. Notably, the German CLL5 trial which enrolled 193 patients with a median age of 70 years has challenged the superiority of purine analogs over chlorambucil in elderly patients.[17] Despite significantly improved response rates with fludarabine, there was no PFS benefit and overall survival tended to be longer with chlorambucil, although this finding did not reach statistical significance.

This IPD analysis includes survival data from 488 elderly patients aged 70 years or over (representing 18% of all individuals enrolled). In this subgroup of patients, purine analogs had less effect on PFS (OR=0.88 compared with 0.70 for age <60 and 0.62 for age 60-69), although the difference between these groups was not significant (*P* trend = 0.1). For overall survival, there was less effect with older age (*P* trend = 0.03) and no evidence of benefit for patients aged 70 years or over. It should also be borne in mind that elderly patients will have been selected as fit enough to receive the trial treatments. Thus, further trials for elderly patients

stratified according to co-morbid conditions, using for example the cumulative illness rating scale (CIRS) as incorporated by the GCLLSG, are needed.

This IPD analysis is also the first meta-analysis examining the benefits of combining purine analogs with cyclophosphamide compared to treatment with single agent purine analogs. Four trials including 1,403 patients were available for this analysis. Combination of purine analogs with cyclophosphamide resulted in significantly improved overall response rates, improved quality of response and longer response duration. Notably, the extent of these beneficial treatment effects was substantially greater than that achievable with purine analogs when compared with alkylator-based treatment (e.g. absolute improvement of PFS at five years of 20% for purine analog/cyclophosphamide combinations over purine analogs alone compared with an absolute PFS benefit at five years of 5% for single-agent purine analogs compared to alkylators). Moreover, this benefit was robust in all subgroups examined (age, sex, stage, beta-2 microglobulin high/low, IGHV mutational status, presence of del(17p13) and del(11q13), respectively).

However, substantially improved responses and PFS achieved with purine analog/cyclophosphamide combinations did not translate into a survival benefit.

Data from 2 trials suggested that cladribine may be more effective than fludarabine, but the number of patients randomized was small and this effect was seen less in the trial which used combination treatment with cyclophosphamide.

Despite the inclusion of a markedly higher number of patients, we were still not able to detect a significant overall survival benefit for first-line treatment with purine analogs in comparison to alkylators. In the CLB-9011 trial, a survival difference emerging only after five years has now been reported,[25] and length of follow up may be an issue. However, the interpretation of overall survival data derived from randomized trials is difficult when there are active second-line treatments available. This methodological problem may be overcome by accounting for the second-line treatments administered, but unfortunately the limited data available on second-line treatments were insufficient for detailed analysis. Given this, with regards to overall sur-

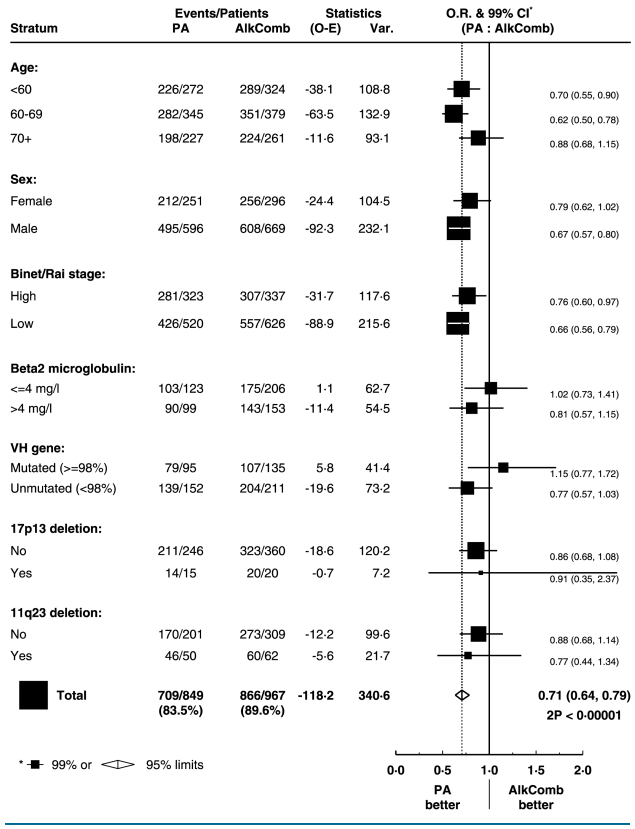
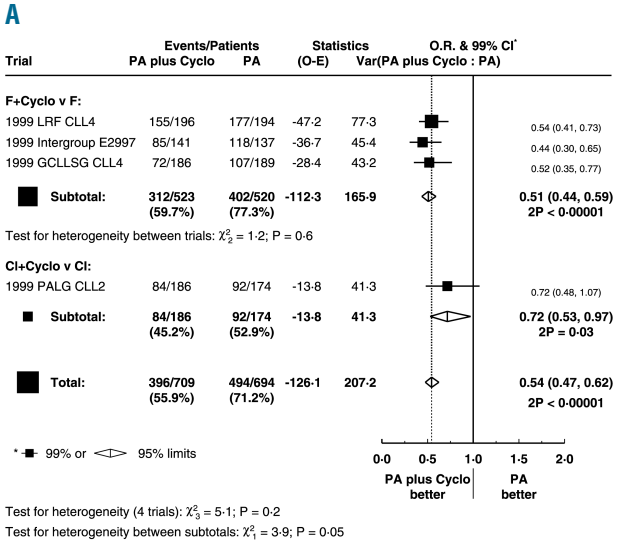


Figure 2. Effect of purine analog *versus* alkylating agent based treatment on progression free survival within subgroups.



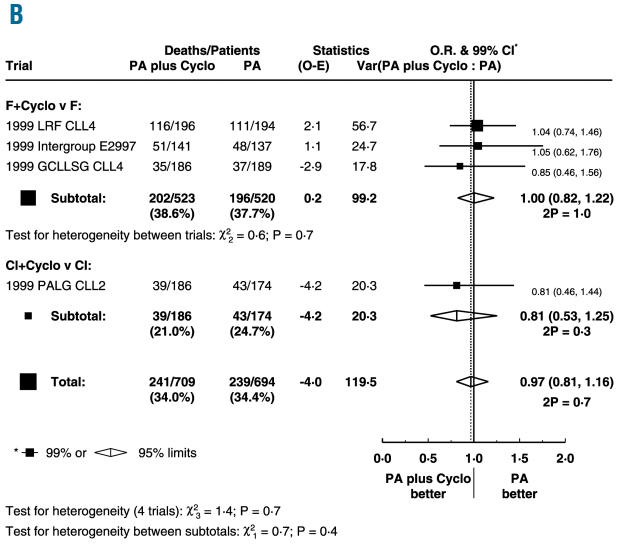


Figure 3. Effect of the addition of cyclophosphamide to a purine analog on (A) progression free survival and (B) overall survival. Format as Figure 1.

vival, the essential clinical question addressed by the trials comparing purine analogs with alkylators was whether giving purine analogs early is better than giving them in later lines of therapy. As effective second-line treatment options such as purine analogs with or without cyclophosphamide cannot be withheld from patients for ethical reasons, most recently or currently conducted CLL trials have chosen PFS as primary end point. Nevertheless, prolongation of overall survival remains the most important measure of clinical benefit for CLL patients. To facilitate the interpretation of survival data in clinical trials, we, therefore, suggest that future protocols should attempt to collect data on second-line treatment, as has recently been proposed by the CONSORT Group in the updated guidelines for reporting parallel group randomized trials.[26]

From a methodological point of view, and in accordance with meta-analyses from tabulated data, this IPD analysis also found a significant degree of heterogeneity between trials for both response and PFS, but not for overall survival. However, the beneficial effect of purine analogs on PFS and DFS was significant using either a fixed effect or random effects method. As discussed earlier, this might be due to overall trial design, alkylator doses applied, response definitions and crossover instructions. Whereas the CLL 101 trial, for example, withdrew patients who were unresponsive after 2 courses, a course of action likely to disfavor alkylating agents which seem to act more slowly, the LRF CLL4 did not require response assessment until six months in the chlorambucil arm, and encouraged continuation to maximum response using up to 12 months of treatment. However, we also found profound differences concerning collection and recording of data. For example, the proportion of patients with a missing response varied enormously between trials, from 0 to 22%, and reasons for the missing responses were not generally recorded. Moreover, the proportion of patients with a missing response who died within six months also varied widely between trials, from 0 to 86%. In addition, we found major differences in the coding of response levels. In particular, only 2 trials coded nodular partial response separately, and although inclusion of these as good response or not did not alter overall conclusions, it did alter the estimated size of treatment effect. Non-standard methods of collection and recording of adverse effects are likely to make results more difficult to interpret, and even IPD meta-analysis unreliable. It would be helpful if consistent methods of response recording could be used in terms not only of response definitions (e.g. using the IWCLL criteria[27]), but also of the 'not assessable' category and exclusions.

The recent introduction of monoclonal antibody treatment into combination therapy regimens for CLL is further improving outcome, and for the first time a trial has reported an overall survival difference[28]. However, even where there may be a difference in survival, usually trials are not large enough and follow up not long enough to detect it. It seems that meta-analysis will continue to be required to establish whether treatments which improve PFS also prolong survival, as well as determining which subgroups benefit. To make this possible, it is important to use consistent definitions and data collection procedures, to extend follow up, and to report fully, taking advantage of the additional space available with electronic publishing.

This review has shown that although purine analog/cyclophosphamide combinations improve PFS, if not survival, for most patients, there may be groups that do not benefit, such as the elderly. Questions remain about dose and duration of all treatments, including chlorambucil.

Appendix

CLLTCG participating groups a*nd trialists*

Appendix: CLLTCG participating groups and trialists

Bayonne, France: M Bauduer; Berlex Oncology, USA: J Gribben; Berlin Free University, Germany: R Herrmann, E Thiel; Cancer and Leukemia Group B (CALGB), USA: K Rai, R Larson; Cardarelli Hospital, Naples, Italy: F Ferrara; CLL Support Association, UK: J Barnard, H Pearce, C Taylor; Cochrane Haematological Malignancies Group: C Brillant, M Steurer, O Weingart; Eastern Cooperative Oncology Group (ECOG), USA: IW Flinn, A Funkhouser, M Tallman, Z Sun; European Organisation for Research on Treatment of Cancer (EORTC): B Jaksic, S Suciu; French Cooperative Group on CLL, France: S Chevret, G Dighiero, M Leporrier; Genta, USA: SR Frankel; Genzyme, USA: C Sirard, P Hillmen, B Trehu, M Felder; German CLL Study Group (GCLLSG), Germany: R Busch, B Eichhorst, M Hallek, S Stilgenbauer; Hellenic Haematological Society, Athens, Greece: G Pangalis; Hospital T. Alvarez, Buenos Aires, Argentina: R Bezares; Dutch-Belgian Hemato-Oncology Cooperative Study Group (HOVON): MHJ van Oers, W van Putten; Italian multicenter group: M Gobbi, M Spriano; Mansoura University, Egypt: M Mabed; Medical Research Council, UK: D Catovsky, S Richards*, R Wade; National Cancer Institute, Egypt: T Abdelhamid; National Cancer Research Institute (NCRI), UK: C Dearden; OSHO/GCLLSG/Intergroup: W Knauf; Polish Adult Leukemia Group (PALG), Poland: J Blonski, K Jamroziak, T Robak; Rome, University La Sapienza, Italy: F Mauro; Schering AG/Inveresk, Germany: W Hiddeman, SA Johnson, G Longthorne;*

Suddeutsche Hamoblastose Gruppe (SHG), Germany: MJ Rummel; Scandinavian/Australian multicenter group: G Juliusson; Tirana, Albania: P Pulluqi; University of Bologna, Italy: PL Zinzani; Università di Trieste, Italy: G Pozzato; US Oncology, Houston, USA: C Reynolds; Weill Medical College of Cornell University, New York, USA: RR Furman; Secretariat: J Durrant, P Elphinstone, V Evans, L Gettins, C Hicks, S James, M Clarke, L MacKinnon, TM McHugh, P Morris, S Read, C Gregory.

** Writing committee*

Authorship and Disclosures

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